CXCV. THE BEHAVIOUR OF *l*-ASCORBIC ACID AND CHEMICALLY RELATED COMPOUNDS IN THE ANIMAL BODY. ANTISCORBUTIC ACTIVITY IN RELATION TO RETENTION BY THE ORGANISM.

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ALTHOUGH *l*-ascorbic acid (*l*-xylo-ascorbic acid) is the most active antiscorbutic substance so far known, other chemically related compounds have been shown to possess antiscorbutic activity. Of these *d*-arabo-ascorbic acid (*d*-erythro-3-ketohexonic acid lactone) [Maurer and Schiedt, 1933] possesses about 1/20 [Dalmer and Moll, 1933; Demole, 1934], *l*-rhamno-ascorbic acid (6-methyl-*l*-arabo-3-ketohexonic acid lactone) 1/5 [Reichstein *et al.*, 1935] of the activity of *l*-ascorbic acid. A homologue *l*-gluco-ascorbic acid is 1/40 active [Reichstein, 1934 and private communication]. This compound was tested recently by the writer and, as will be seen from Fig. 1, was found to be endowed approximately

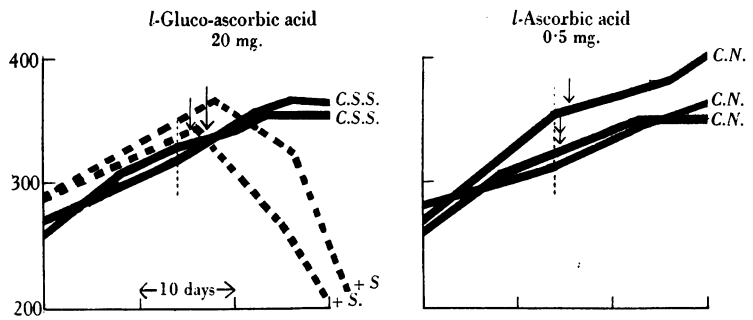

Fig. 1.

+ = Died
C. = Chloroformed.
S. = Scurvy.
S.S. = Slight scurvy.
N. = Normal.
| = Beginning of dosing.
↓ = Onset of clinical symptoms of scurvy.
■■■ = Negative control.

with the activity claimed for it. Unfortunately, owing to scarcity of material, a more accurate assessment was not carried out.

In contradistinction to the above compounds *d*-ascorbic acid failed to show antiscorbutic activity when administered to guinea-pigs on a scorbutic diet in daily doses of 20 mg. [Demole, 1934]. *d*-Gluco-ascorbic acid (*d*-3-ketogluco-heptonofuranolactone) [Ault *et al.*, 1933] and *d*-galacto-ascorbic acid (*d*-3-keto-galactoheptonofuranolactone) [Baird *et al.*, 1934] were found to be totally inactive in daily doses of 10 mg. (Zilva, unpublished results).

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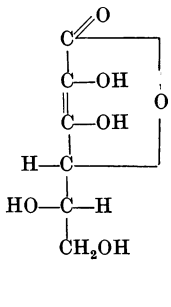
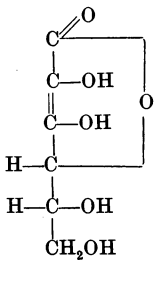
It will be seen from formulae I–VII that in all the compounds which show antiscorbutic activity the ring engages the hydroxyl group to the right of the carbon chain and that the opposite is the case with the inactive compounds. In consequence Reichstein [1934] and Haworth [1934] tentatively suggested that such configuration may be a necessary condition of antiscorbutic activity in these substances.



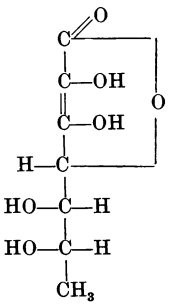
l-ascorbic acid

I



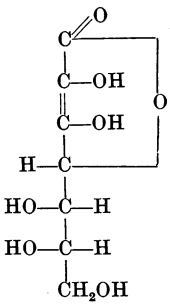
d-arabo-ascorbic acid

II



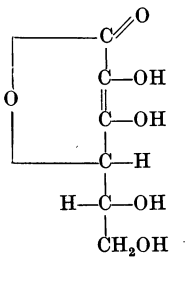
l-rhamno-ascorbic acid

III



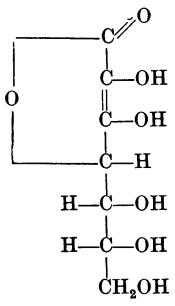
l-gluco-ascorbic acid

IV



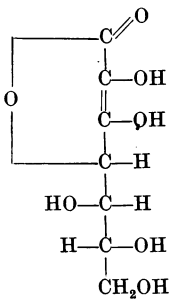
d-ascorbic acid

V



d-gluco-ascorbic acid

VI



d-galacto-ascorbic acid

VII

In this communication data are produced which promise to become criteria of value in fathoming the specificity and general mechanism of the biological action of the ascorbic acid group of compounds. The results show that when the animal organism (guinea-pig) is exhausted of *l*-ascorbic acid and the above substances are introduced into the system, the degree of "fixation" by the tissues, especially by those which show "selective fixation" such as the adrenals, anterior lobe of the pituitary, intestine *etc.*, is controlled by the degree of antiscorbutic activity. This fact was reflected further by the excretion of these compounds by the kidney in amounts which varied inversely with the potency.

Technique.

The purity of the various compounds was assessed by titration with indo phenol. With the exception of a few intraperitoneal and intracardiac injections, the partially neutralised acids were introduced directly into the blood stream through the jugular vein which was layed open under novocaine. After injection the guinea-pigs were placed in a metabolism cage, thus enabling the urine to be

quantitatively collected. Each delivery, except the night urine, was titrated immediately after passing with indophenol at p_H 2·5. Twenty-four hours after injection the animals were killed by stunning and bleeding and the tissues were at once worked up with the utmost speed for analysis. With the exception of the pituitary the material was extracted twice with trichloroacetic acid by grinding with sand and centrifuging, after which the extracts were titrated with $N/1000$ indophenol at p_H 2·5. The pituitary was removed immediately after killing, placed in a 2 % solution of normal lead acetate for about a minute and then introduced into 0·4 % $AgNO_3$ and kept in this solution in the dark for 15 minutes. At the end of this time the preparation was washed, fixed with sodium thiosulphate and preserved in 50 % alcohol. The degree of darkening of the anterior lobe was graded from 0 to ++++. In the case of the "carcass", the skinned body of the animal from which the brain and the internal organs were removed was passed quickly through a mincing machine, well mixed, and an aliquot portion (20 g.) was extracted with trichloroacetic acid as mentioned above. The guinea-pigs on the mixed diet received a liberal supply of cabbage.

Discussion of results.

In appraising the experiments it is convenient to consider first the results obtained with the guinea-pigs which received no injection on a scorbutic diet. Such animals, as has been shown by De Caro [1934], are soon depleted of their store of *l*-ascorbic acid. It will be seen that the negative control group lost most of the vitamin contained in the tissues. It is indeed doubtful whether the reduction of the indicator in this case is due mainly to *l*-ascorbic acid. The residual reducing capacity of the tissues of the control animals, whatever its significance, has, however, to be taken into consideration when the figures in the other groups are discussed.

The guinea-pigs which received 50 mg. of *l*-ascorbic acid show a distribution of the injected vitamin amongst the tissues similar to that observed in the case of guinea-pigs kept on a mixed diet. The same "selective fixation" is observed in the adrenals, anterior lobe of the pituitary, intestine and liver as is usually found in all animals whether they obtain the vitamin from a sufficient intake in the food or by synthesis. In addition, with the possible exception of the adrenals and liver, there was no very marked disparity between the quantities of *l*-ascorbic acid found in the respective tissues of the guinea-pigs of the two groups.

Reference must now be made to the animals which received the inactive compounds or rather the compounds which have no antiscorbutic activity in doses so far tested, namely, *d*-gluco-ascorbic acid and *d*-galacto-ascorbic acid. In these cases, considering the limitation of the method, the figures appear to be identical with those of the negative control animals. In other words, neither of these compounds has been "fixed" in the tissues, at least in the reduced form.

This observation becomes arresting when other results in the table disclose the fact that *d*-arabo-ascorbic acid (1/20 active) fills an intermediate position in this respect between the fully active *l*-ascorbic acid and the "inactive" *d*-gluco-ascorbic and *d*-galacto-ascorbic acids. This evidence strongly suggests that the antiscorbutic activity of this class of compounds is correlated with their "fixation" in the tissues.

The weakest link in this chain of evidence is the behaviour of *l*-gluco-ascorbic acid. The quantities of this compound which were "fixed" differed from those of the "inactive" compounds by amounts which fall within the limits of experimental error. It is appropriate, however, to point out at this juncture that

Table I.

Compound injected	Quantity injected mg.	Wt. of animal g.	Scorbutic diet days	Quantity found: Small intestine mg./g.	Small intestine Total mg.	Large intestine mg./g.	Large intestine Total mg.	Liver mg./g.	Liver Total mg.	Adrenal mg./g.	"Carcass" mg./g.	"Carcass" Total mg.	Pituitary	Excreted in urine during 24 hours mg.
l-Ascorbic acid	50	272	7	0·15	2·0	0·09	1·3	0·13	2·2	0·7	—	—	+ + +	13
	50	300	5	0·11	1·7	0·08	1·0	0·14	1·8	0·8	0·03	4·0	+ + + +	13
(*l*-Xylo-ascorbic acid)	50	300	6	0·12	1·7	0·12	1·3	0·20	2·5	1·0	0·04	5·8	+	12
	50	295	6	0·15	2·1	0·10	1·1	0·16	2·1	1·1	0·04	5·3	+ + +	13
	50	280	7	0·17	2·1	0·11	1·2	0·17	2·1	0·7	0·04	5·2	+	13
	50	292	20	0·12	1·9	0·10	1·1	0·21	2·9	0·9	0·04	4·6	+ + + +	14
	50	295	20	0·11	1·6	0·07	0·9	0·16	1·9	0·3	0·03	3·8	+ to + +	11
	50	295	8	0·16	2·1	0·10	1·2	0·17	1·9	0·6	0·03	4·9	+ + + to + + + +	10
d-Arabo-ascorbic acid	45	275	6	0·07	0·9	0·05	0·7	0·09	1·1	0·5	0·00	0·0	trace to +	14
	45	285	7	0·08	1·0	0·07	1·0	0·11	1·3	0·3	0·02	3·0	trace	16
	55	335	6	0·10	1·3	0·03	0·4	0·09	1·2	0·1	0·02	2·8	0 to trace	17
	55	300	6	0·08	1·0	0·05	0·6	0·11	1·0	0·2	0·02	3·8	0 to trace	19
	45	310	6	0·11	1·3	0·06	0·7	0·10	2·0	0·7	0·03	4·7	+ +	19
	45	350	6	0·09	1·2	0·05	0·7	0·10	1·3	0·1	0·01	2·7	+ +	10
	50	275	6	0·12	1·3	0·08	0·9	0·10	0·9	0·1	0·03	3·8	+	15
	50	305	7	0·07	1·0	0·07	0·8	0·06	0·9	0·5	0·02	3·0	+ + +	19
d-Galacto-ascorbic acid	50	325	6	0·08	1·2	0·02	0·3	0·07	1·0	0·5	0·02	3·3	0 to trace	25
	50	315	6	0·04	0·6	0·02	0·2	0·07	0·9	0·2	0·01	1·5	0	28
	50	300	6	0·05	0·6	0·02	0·2	0·05	0·6	0·0	0·00	0·0	0 to trace	23
	50	290	7	0·06	0·6	0·03	0·3	0·07	0·6	0·0	0·00	0·0	0 to trace	26
	50	280	7	0·03	0·3	0·01	0·1	0·05	0·5	0·0	0·00	0·0	0 to trace	21
d-Gluco-ascorbic acid	55	300	6	0·07	0·9	0·03	0·4	0·08	1·2	0·0	0·00	0·0	trace	20
	55	305	6	0·06	0·8	0·05	0·7	0·06	0·8	0·0	0·00	0·0	0 to trace	26
	55	290	6	0·07	0·8	0·02	0·2	0·09	1·0	0·3	0·00	0·0	0	39
	55	310	7	0·03	0·4	0·03	0·3	0·05	0·6	0·0	0·00	0·0	0 to trace	34
	50	270	6	0·05	0·7	0·03	0·3	0·05	0·6	0·0	0·00	0·0	0	30
	50	300	7	0·04	0·6	0·04	0·5	0·04	0·5	0·0	0·01	1·6	trace	26
	50	275	7	0·05	0·7	0·05	0·5	0·05	0·6	0·0	0·00	0·0	0	26
l-Gluco-ascorbic acid	50	320	6	0·06	0·8	0·04	0·4	0·09	1·2	0·4	0·00	0·0	0	17
	50	315	5	0·09	1·2	0·06	0·8	0·07	1·0	0·2	0·01	2·0	0	21
	50	330	6	0·05	0·9	0·04	0·7	0·06	1·0	0·2	0·01	1·5	0	23
	50	300	6	0·06	0·8	0·03	0·4	0·07	1·0	0·2	0·02	2·3	0	23
None	0	265	5	0·05	0·7	0·02	0·2	0·10	1·0	0·2	0·00	0·0	0 to trace	—
	0	272	7	0·04	0·7	0·03	0·5	0·04	0·8	0·0	0·00	0·0	0	—
	0	280	8	0·04	0·4	0·02	0·3	0·07	0·8	0·0	0·00	0·0	trace to +	—
Mixed diet	0	260	0	0·20	2·0	0·10	1·5	0·22	2·2	1·6	0·04	5·0	+ + to + + +	—
	0	280	0	0·17	2·5	0·10	1·2	0·21	3·2	1·1	0·04	5·9	+ + + +	—
	0	260	0	0·14	2·2	0·10	1·3	0·40	6·0	1·1	0·04	5·5	+ + + +	—

l-gluco-ascorbic acid is claimed to be only 1/40 active and that my test suggests that it may even be less potent. Other results, to be discussed later, support the view of "fixation" by the tissues even in this case.

The contrast between the behaviour of the various compounds is more revealing when the urinary excretions of the acids are compared. Although the greater part of such excretion as took place was completed during approximately the first four hours after injection, it was thought advisable to continue the determination in the urine for twenty-four hours. After this time the amount thus voided became hardly appreciable. It is seen from the figures that the quantity of the compounds passed in the urine is the inverse of the degree of the antiscorbutic activity, a fact which is partly explained by the differential "fixation" of the various acids by the tissues. Moreover, the difference between the amount of *l*-gluco-ascorbic acid excreted in the urine on the one hand and of *d*-gluco-ascorbic acid and *d*-galacto-ascorbic acid on the other appears to fall outside the limit of experimental error, particularly when it is borne in mind that greater accuracy is obtained in titration of these compounds in urine than in most of the tissues discussed here. There is, therefore, an indication, despite its very low activity, that *l*-gluco-ascorbic acid is also "retained" more efficiently than the "inactive" compounds. It must, nevertheless, be acknowledged that this point calls for further examination.

To the writer it seems that the general results justify the assumption that the antiscorbutic activity of these chemically related compounds is connected with their capacity of being "retained" by the tissues of the animal organism. It is obvious that the continuation of this investigation, which is pregnant with a number of possibilities, will shed more light on the matter. The advance of the problem is at the moment slow, being to a great extent dependent upon the continued supply of synthetic material.

With the exception of *l*-ascorbic acid, for which I am indebted to Messrs. Hoffmann La Roche, Ltd., the compounds used in this inquiry were prepared at the University of Birmingham by Prof. W. N. Haworth, Dr E. L. Hirst, Mr J. K. N. Jones and Mr F. Smith for the purpose of assessing the antiscorbutic activity. I should like to express my gratitude to my colleagues for offering me the opportunity of utilising this valuable material in the present work.

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